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(54) Title: IMMUNOGLOBULIN A1 PROTEASES (IgA1 PROTEASES), METHOD OF GENE-TECHNOLOGICALLY PRODUCING THIS TYPE OF ENZYME AND VACCINES CONTAINING THE ENZYMES AND FRAGMENTS THEREOF FOR IMMUNIZING AGAINST BACTERIAL MENINGITIS AND OTHER DISEASES CAUSED BY IgA1 PROTEASE-PRODUCING BACTERIA

(57) Abstract

Secretion of IgA1 proteases is a characteristic of Haemophilus influenzae and several other bacterial pathogens causing infectious diseases, including meningitis. Indirect evidence suggests that the proteases are important virulence factors. The iga gene encoding IgA1 protease, especially that from H. influenzae serotype b, has been cloned into Escherichia coli in which the recombinant iga gene was expressed and the resulting protease secreted. Sequencing a part of a 7.5 kb DNA fragment containing the iga gene revealed a large open reading frame (ORF) with a strongly biased codon usage and having the potential of encoding a protein of 1541 amino acids and a molecular mass of 169 kd. Putative promoter and terminator elements flanking the ORF have been identified. Comparing the deduced amino acid sequence of the produced H. influenzae IgA1 protease with that of a similar protease from Neisseria gonorrhoeae reveals several domains with a high degree of homology. Analogous to mechanisms known from the N. gonorrhoeae IgA protease secretion, there is proposed a scheme of post-translational modifications of the H. influenzae IgA1 protease precursor leading to a secreted protease with a molecular mass of 108 kd which is close to the M_r 100 kd estimated for the mature IgA1 protease.

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Immunoglobulin A1 proteases (IgA1 proteases), method of gene-technologically producing this type of enzyme and vaccines containing the enzymes and fragments thereof for immunizing against bacterial meningitis and other diseases caused by IgA1 protease-producing bacteria

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The present invention relates to immunoglobulin A1 proteases (IgA1 proteases) and fragments thereof and also to a method of gene-technologically producing the inventive IgA1 protease by cloning the gene for the IgA1 protease in question from a micro-organism, in particular Haemophilus influenzae serotype b, in an E. coli host organism from which the IgA1 protease formed is secreted extracellularly. The inventive IgA1 proteases can be used in vaccines, in particular for the prevention of meningitis, but also for immunizing against allergic diseases, gonorrhoea and other diseases caused by IgA protease-producing bacteria.

Background of the invention:

The immunoglobulin A1 proteases (IgA1 proteases) are extracellular bacterial enzymes which specifically cleave the heavy chain of human IgA1 molecules in the hinge region. Production of IgA1 protease seems to be correlated with the ability of the bacteria to infect and in some instances invade mucosal membranes. Notably, the three leading causes of bacterial meningitis (Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae) secrete IgA1 proteases whereas closely related non-pathogenic species are devoid of similar enzyme activity. IgA1 protease production is also a property of certain bacterial species causing vaginal and urinary tract infections, such as Neisseria gonorrhoeae and Ureaplasma urealyticum. In addition, several oral bacteria

involved in dental plaque formation (Streptococcus sanguis, Streptococcus oralis, and Streptococcus mitis biovar 1 (the two latter species are former parts of Streptococcus mitior) and in the pathogenesis of periodontal diseases (Bacteroides and Capnocytophaga species) secrete IgA1 proteases (Kilian, M. and Reinholdt, J.: "Interference with IgA defence mechanisms by extracellular enzymes", p. 173-208 in C.S.F. Easmon and J. Jeljaszewics (ed.), 1986, Medical Microbiology vol. 5, Academic Press, Inc., London; see also Mulks, M. H.: "Microbial IgA proteases" p. 81-104 in I. A. Holder (ed.), 1985, Bacterial Enzymes and Virulence, CRC Press, and Plaut, A.G., Ann. Rev. Microbiol. 37, 603-622 (1983)). IgA1 proteases are believed to be important virulence factors as they allow bacteria to interfere with the protective mechanisms of IgA1, which is the predominant immunoglobulin class on relevant mucosal surfaces (Kett, K. et al., J. Immunol. 136, 3631-3635 (1986)).

Because of the specificity of IgA1 proteases for human IgA1 experiments with animals cannot confirm the pathogenic significance of these enzymes. However, extensive cleavage of IgA in secretions and other body fluids from patients infected with said bacteria is observed. Furthermore, it has been shown that nasopharyngeal secretions from children with a history of allergic diseases, more than from healthy children, show extensive IgA1 protease-induced degradation of the IgA, which was to form a protective barrier to potential allergens and microorganisms (Christensen, C.H. and Kilian, M., Acta Path. Microbiol. Immunol. Scand. 92C, 85-87, 1984).

Several enzymatically different types of IgA1 proteases have been reported, each cleaving the human IgA1 molecule at a specific site in the hinge region, as shown in fig. 1 (Kilian, M. and Reinholdt, J., supra). In Haemophilus

influenzae at least two such distinct enzymes are known (type 1 cleaving the PRO-SER paptide bond at position 231-232 and type 2 cleaving the PRO-SER peptide bond at position 235-236). Likewise, Neisseria meningitidis and Neisseria gonorrhoeae may secrete a type 1 or a type 2 protease (see fig. 1).

Polymorphism of IgA1 proteases:

IgA1 proteases from different bacteria have been compared by genetical and serological methods. Hybridization experiments using the bulk of a gonococcal IgA1 protease gene as probe revealed substantial homology (>78%) between IgA1 protease genes from different gonococcal and meningococcal strains. More surprisingly, an estimated 67-75% homology between the gonococcal gene and that from a Haemophilus influenzae strain (Rd) was observed (Koomey, J.M. and Falkow, S., Infect. Immun. 43, 101-107, 1984). However, the use of restriction enzyme mapping of IgA1 protease genes revealed a considerable degree of polymorphism even between strains belonging to the same species (Halter, R. et al. EMBO J. 3, 1595-1601, 1984; Bricker, J. et al., Infect. Immun. 4, 370-374, 1985; Mulks, M.H. and Knapp, J.S., Infect. Immun. 55, 931-931, 1987). Within a large collection of Haemophilus influenzae serotype b strains four different IgA1 protease gene types were detected with restriction enzyme analyses (Poulsen, K., Hjorth, J.P. and Kilian, M. Infect. Immun. 56, 987-992, 1988).

The extensive polymorphism has been confirmed by comparing IgA1 proteases with neutralizing antibodies raised in rabbits. When immunized with IgA1 protease preparations rabbits respond with antibodies capable of neutralizing the enzyme used for immunization. With such antibodies at least 15 antigenically different types of IgA1 proteases

have been identified among H.influenzae isolates (Kilian, M. et al., Molec. Immunol. 20, 1051-1058, 1983) and two types among H.influenzae serotype b (Poulsen K. et al. supra). A partial antigenic relationship has been observed between one out of two strains of N.meningitidis and one strain of N.gonorrhoeae (Kilian, M., et al., Ann. N.Y. Acad. Sci. 409, 612-624, 1983). Furthermore, type 1 and type 2 IgA1 proteases of Neisseiae can be distinguished on the basis of their relative inactivation by sera from patients recovering from meningococcal infection by either type 1 or type 2 protease producing bacteria (Stafford and Plaue, Abstr. Annu. Met. Am. Soc. Microbiol. 1982, B125, p. 38).

Cloning of IgA1 protease genes

The genes encoding IgA1 protease (iga genes) produced by Neisseria gonorrhoeae type 1 (Fishman, Y., Bricker, J., Gilbert, J.V., Plaut, A.G. and Wright, A.: "Cloning of the type 1 immunoglobulin A1 protease from Neisseria gonorrhoeae and secretion of the enzyme from Escherichia coli", 1985, p. 164-168 in G. K. Schoolnik (ed.), The pathogenic Neisseria, Am. Soc. Microbiology, Washington DC) and type 2 (Koomey, J. M., Gill, R.E. and Falkow, S., Proc. Natl. Acad. Sci. USA, 79, 7881-7885, 1982; Halter, R., Pohlner, J. and Meyer, T.F., EMBO, J. 3, 1595-1601, 1984), Haemophilus influenzae type 1 of serotype d origin (Bricker, J. et. al., Proc. Natl. Acad. Sci. USA, 80, 2681-2685, 1983; and Koomey, J. M. and Falkow, S., supra) and type 2 of serotype C origin (Grundy, J. F. et. al., J. Bacteriol. 169, 4442-4450, 1987) Neisseria meningitidis (Koomey and Falkow, supra and Streptococcus sanguis (Gilbert, J.V. et al., Infect. Immun. 56, 1961-1966 (1988)) have been cloned.

E. coli transformed with each of these iga genes express

IgA1 protease activity and the E. coli hosts, which harbour the H. influenzae and the N. gonorrhoeae iga genes, have been found to excrete the protease. At present, an example of the type 2 IgA1 protease gene from N. gonorrhoeae is the only iga gene for which the nucleotide sequence is known (Pohlner, J. et al., Nature 325, 458-462 (1987); see also DE OS 36 22 221). The deduced primary structure of the protein together with analyses of intermediate IgA1 protease precursors have revealed that the protease is expressed as a preprotein containing a signal peptide, the structural protease and a helper protein, which is autoproteolytically processed during the secretion process. Despite the similarity between the enzymes from Neisseria sp. and H.influenzae it has been concluded that the Haemophilus enzyme is not transproted by the mechanism used for secretion of the Neisseria IgA1 protease (Fishman et. al., supra; see also DE OS 36 22 221).

It has now surprisingly been found that the gene for IgA1 protease from Haemophilus influenzae can be cloned in E. coli to achieve extracellular enzyme secretion, thus making it possible to extract the enzyme from the cultivation medium.

Previously, fragments of the cloned type 1 IgA1 protease gene of H. influenzae serotype d origin have been used as probes in Southern blot experiments to study the restriction site polymorphism of the iga gene among different H. influenzae serotype b strains (Poulsen, K., Hjort, J.P. and Kilian, M., Infect. Immun. 56 987-992 (1988)). Based upon these iga gene restriction patterns the strains could be divided into four groups, which correlated with previously observed clusters of multilocus genotypes (electrophoretic types). Three of the iga gene restriction types, which appeared to represent 98% of the

H. influenzae serotype b population, exclusively contained strains of the same unique IgA1 protease "inhibition type" and therefore could form the basis for the development of a vaccine against H. influenzae meningitis.

Experiments with inhibitory antibodies have shown that the three gene types direct the production of proteases with a mutual epitope, which is found in each of the three proteases.

Detailed Description of Aspect 1

In the present invention H. influenzae strain HK368 was chosen as representative for the most common serotype b iga gene type (Poulsen, K. et al., supra). In the following the cloning and sequencing of the iga gene from this strain is described. Comparing the deduced amino acid sequence of this protease to that of N. gonorrhoeae reveals interesting regions of homology.

In the following the invention is illustrated in more detail, reference being made to the drawing in which

fig. 1 shows the primary structure of the hinge region of human IgA1;

fig. 2 shows the structure of the H. influenzae serotype b strain HK368 IgA1 protease gene;

fig. 3 shows the nucleotide sequence of the cloned iga gene from H. influenzae serotype b strain HK368;

fig. 4 illustrates the amino acid homologies between the deduced H. influenzae iga gene product and the N. gonorrhoeae iga gene product, and

Fig. 5 shows a physical map of lambda HF13iga-1 expressing the meningococcal IgA1 protease gene from strain HF13.

The invention is illustrated in more detail in the following. When carrying out the experiments the following materials and methods were used:

Bacterial strains. The wild-type H. influenzae serotype b strain HK368 isolated from cerebrospinal fluid from a meningitis patient was the source of whole-cell DNA for molcular cloning. It was grown in Brain Heart Infusion broth (Difco, Detroit, Mich.) supplemented with hemin and nicotinamide adenine dinucleotide (NAD) (Kilian, M. J. Gen. Microbiol. 93, 9-62 (1976)).

E. coli strain K802 was used for propagation of lambda phages. The M13mp19 phage and recombinant derivatives hereof were propagated in E. coli JM109 as described by Yanisch-Perron, C. et al., Gene 33, 103-119 (1985).

Enzymes and chemicals. Restriction endonucleases were purchased from Amersham International (Amersham, England) and Boehringer GmbH (Mannheim, FRG). T4 ligase, Proteinase K, RNase A, and DNase I were obtained from Boehringer; DNA polymerase I, DNA polymerase I Klenow fragment, and radioactively labelled deoxynucleotides were from Amersham. Exonuclease III came from Pharmacia (Uppsala, Sweden), lysozyme from Sigma (St. Louis, MS), M13 pentadecamer primer from New England Biolabs (Beverly, CA), and Sequenase DNA sequencing kit was from United States Biochemical Corporation (Cleveland, OH).

Cloning of H. influenzae HK 368 DNA in phage λL47.1. DNA from H. influenzae isolated as described previously

(Poulsen, K. et al., supra) was partially digested with Sau3A and fractionated by agarose gel electrophoresis. Fragments ranging in size from 12 to 20 kilobases (kb) were extracted from the gel by electroelution and cloned using λL47.1 as BamHI substitution vector.

Phages packaged in vitro as described by Maniatis et al., "Molecular Cloning. A laboratory Manual", Cold Spring Harbor Laboratory, N.Y., were plated on E. coli strain K802 and positive plaques identified by in situ hybridization. Positive plaques were purified by replating on E. coli strain K802 and DNA isolated from 20 ml phage cultures (Mikkelsen, B.M. et al., Biochem. Genet. 23, 511-524 (1985)).

Nucleic acid hybridizations and DNA manipulations. As label in the hybridizations (α-^{32}P)-dATP, which was incorporated into the DNA probes by nick-translation, was used (Rigby, P.W.J. et al., J. Mol. Biol. 11, 237-251 (1977)). The in situ probing of phages fixed on nitrocellulose filters was carried out as described by Benton and Davies, Science 196, 180-182 (1977) and Southern blot experiments were performed as described previously (Poulsen, K. et al., supra). Fragments of plasmid pVD116 containing H. influenzae type d iga gene were used as probes. Subcloning into M13mp19 of restriction fragments isolated by electroelution and DNA purifications and manipulations were carried out as described by Maniatis et al., supra.

Progressive deletions of recombinant M13mp19 phages for the sequencing procedure were produced by varying the time length of Exonuclease III digestion of BamHI/SacI opened replicative form DNA (Yanisch-Perron et al., supra). For removal of the resulting single stranded ends S1 nuclease was used instead of Exonuclease VII.

DNA sequencing. The sequences of individual clones were determined by the dideoxynucleotide chain termination method (Sanger, F. et al., Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977) and Biggin, M.D. et al., ibid. 80, 3963-3965 (1983)) using the 15-mer universal primer and (α-^{35}S)-dATP. For some of the sequences Sequenase DNA sequencing kit was used according to the manufacturer's recomendations. The program of Larson and Messing (Nucl. Acids Res. 10, 39-49 (1982)) was used to assemble to sequence data using an Apple IIe computer.

Sequence alignment. Computer assisted analysis of amino acid sequence data was performed by the program described in J. Mol. Biol. 195, 43-61 (1987).

Assay for IgA1 protease activity. IgA1 protease activity in phage lysates was detected by mixing 1 vol. of the lysate supernatant with 1 vol. of a 2 mg/ml solution of hyman myeloma IgA1. After overnight incubation, cleavage of the substrate IgA1 was demonstrated by immunoelectrophoresis as described by Kilian, M. et al., Mol. Immunol. 220, 1051-1058 (1983)).

Immunization and inhibition assay. The supernatant of a E. coli lysate of a recombinant phage containing the iga gene was used for immunization of a rabbit. The immunization procedure and protease inhibition assay was as described previously (Kilian, M. et al., supra).

The following results were obtained:

Cloning of the H. influenzae serotype b iga gene in E. coli.

The E. coli plasmid pVD116 contains the iga gene from H.

influenzae strain Rd^-/b^+ (Koomey, J. M., Proc. Natl. Acad. Sci. USA 79, 7881-7885 (1982)). A 2.0 kb ClaI/PstI fragment of this plasmid containing the 5' end of the iga gene of serotype d origin was used as radioactively labelled probe to isolate the iga gene from a lambda-phage library of H. influenzae serotype b strain KH368 DAN using the vector λL47.1. Replica filters of approximately $2x10^{-3}$ recombinant λ-phages from the non-amplified library were screened with the probe. Sixteen positive clones labelled λ368iga-1 to λ368iga-16 were found. Assuming that iga is a single copy gene, this frequency is in acceptable agreement with the estimated H. influenzae genome size of $1.8x10^{-3}$ kb and an average insert of 15 kb in the recombinant λ-phages. Eleven of these positive clones were purified and recombinant phage DNA isolated. The localization of recognition sites for the restriction endonucleases EcoRI and HindIII in the insert DNA were determined by complete and partial digestions of DNA from the individual λ368iga phages as previously described (Mikkelsen, B.M. et al., supra). In addition, DNA from λ368iga-8 and λ368iga-16 were mapped with respect to the restriction enzymes BamHI, ClaI, and PstI. The restriction maps of the inserts overlap are shown in Fig. 2. This strongly suggests that the iga gene in H. influenzae strain KH368 is a single copy gene.

Localization of the iga gene. The previously described 5'-iga specific probe together with a 3'-iga specific 2.8 kb PstI/EcoRI fragment of pVD116 were hybridized to replica Southern blots of EcoRI, HindIII, and Sau3A restricted DNA from each of the eleven λ368iga clones analysed. Thereby, the iga gene was deduced to be localized within a 7.5 kb EcoRI/HindIII fragment and oriented as shown in Fig. 2.

The restriction enzyme Sau3A sites at the insert vector junctions are preserved during the cloning. For seven of

the eleven recombinant clones analysed the size and number of DNA fragments generated by Sau3A and hybridizing to the two iga probes were identical. The same pattern of bands was observed in Southern blots of Sau3A digested whole-cell DNA from H. influenzae strain HK368 hybridized with the same two probes (data not shown). This indicated that not rearrangements of the hydbirizing region in these lambda phages had occurred during the cloning procedure. In addition, this result confirms that the same iga-specific sequence had been cloned in these phages.

Liquid lysates from these seven recombinant phage clones were found to possess IgA1 protease activity. This confirms the cloning of the iga gene. In the lysates from three of the eleven clones, i.e. λ368iga-2, λ368iga-4, and λ368iga-5, IgA1 cleaving activity could not be detected. DNA from these three clones lacked some of the Sau3A fragments found in strain KH368 genomic DNA, which hybridized to the 3'-iga probe, and therefore only contain the 5' part of the iga gene (see Fig. 2). The clone λ368iga-8, which encodes IgA1 protease activity, lacked some of the HK368 genomic Sau3A fragments hydbidizing to the 5'-iga probe. This indicates that the plasmid pVD116, which was used as probe, contains sequences originating from the H. influenzae serotype d genome beyond the iga gene.

The 7.5 kb EcoRI/HindIII fragment of λ368iga-16, which contains the iga gene, was subcloned into M13mp19. In the supernatant of liquid cultures of E. coli JM109 transformed with this recombinant M13 phage we detected IgA1 protease activity at a level comparable to the IgA1 cleaving activity found in the supernatant of H. influenzae strain HK368 cultures. Because the iga gene was cloned in the opposite direction of the lac Z gene in M13mp19 this result confirms that the H. influenzae iga

gene is expressed in E. coli. It also indicates that the secretion mechanisms of the IgA1 protease function in E. coli since the M13 phages do not lyse the bacterial cells.

Crude extract from a λ368iga-16 lysate was used to immunize rabbits. The immunoglobulin fraction of the resulting antisera had complete inhibitory effect on the IgA1 protease activity from H. inluenzae strain HK368 culture supernatant. This shows that H. influenzae strain HK368 does not secrete any IgA1 protease unrelated to the one encoded by the iga gene cloned in λ368iga-16.

Nucleotide sequence of the iga gene. The 7.5 kb EcoRI/HindIII fragment of λ368iga-16, which contains the iga gene, occasionally was unstable when subcloned into M13mp19 and propagated in E. coli JM109. Therefore, in the sequencing strategy the internal PstI site was used to divide this fragment (see Fig. 2). The sticky ends of the 5.9 kb HindIII/PstI and the 1.6 kb PstI/EcoRI fragments of λ368iga-16 (Fig. 21) were made blunt ended by the Klenow enzyme and subcloned in both orientations into the HincII site of M13mp19. Deletion derivatives of these subclones were generated by nuclease Exo III digestion for various times of SacI/BamHI-opened replicative form DNA followed by nuclease S1 treatment and self ligation. The 5091 nucleotide (nt) sequence of the 7.5 kb EcoRI/HindIIII fragment presented in Fig. 3 was obtained by sequencing a total of 79 such deletion clones, which gave overlapping sequences of both strands in this central part of the fragment. The sequence across the internal PstI site, i.e. at nt 4856 to nt 4861 in Fig. 3 was verified by subcloning into M13mp19 and sequencing 300 nucleotides from the 3' end of the 1640 base pair HhaI fragment ranging from nt 3349 to nt 4988.

The sequence revealed a large open reading frame (ORF)

with homology to the N. gonorrhoeae iga gene sequence previously published (Pohlner, J. et al, Nature 325, 458-462 (1987)). The suggested iga gene from H. influenzae strain HK368 starting at the first ATG in the ORF as shown in Fig. 3 consists of 4646 nucleotides, including the TAA stop codon, encoding a deduced protein of 1541 amino acids. This primary translation product has a deduced molecular mass of 169 kd in contrast to the estimated size of the mature IgA1 protease of approximately 100 kd. In the discussion below is suggested a scheme for post-translational modifications of the preprotein to account for this discrepancy.

In connection with the present invention the iga gene encoding the IgA1 protease from the H. influenzae serotype b strain HK368 has been cloned and sequenced in order to characterize the protein further and thereby to achieve a better understanding of its role during the bacterial infection. The iga nucleotide sequence presented in Fig. 3 and the deduced primary structure of the IgA1 protease protein reveal several interesting features.

Potential start and stop signals for transcription and translation. The observed expression in E. coli implies that the H. influenzae iga gene must have transcription and translation signals which are recognized by the E. coli cell. A potential ribosome binding Shine-Dalgarno sequence 5'-TAAAGA -3' (nt 248 to nt 253 in Fig. 3) can be found eight nucleotides upstream from the putative translation start at the first ATG in the open reading frame (A at nt 262 in Fig. 3). This sequence has a homology of 5 identical positions out of 6 to the complementary sequence of the 3' end of the E. coli 16 S ribosomal RNA (Shine, J. and Dalgarno, 1, Proc. Natl. Sci. USA 71, 1342-1346 (1974)). In addition, it is located in an acceptable position according to the rules for gene

beginnings suggested by Stormo et al., Nucl. Acids Res. 10, 2971-2996 (1982)). The sequence 5'-TAAACT-3' (nt 235 to nt 240) and 5'-TTGTG-3' (nt 211 to nt 215) might constitute the transcription signals at -10 and -35, respectively. The proposed -10 sequence is identical to the E. coli consensus sequence at the four best conserved positions (Hawley, D.K. and Mc Clure, W.R., Nucl. Acids Res. 11, 2237-2255 (1983)).

Located 19 base pairs upstream from this, the -35 sequence is within the allowed spacing and has a homology of 3 out of 5 positions identical to the E. coli consensus sequence (Hawley, D.K. et al., supra). The identification of these three putative promotor elements strongly support the suggestion of translational initiation at nt 262.

The ORF is ended by two sequential TAA stop codons at nt 4885 to nt 4890. A sequence similar to a typical Rho-independent transcription terminator is found at nt 4919 to nt 4943 and contains a perfect invented repeat with the potential of forming a "hairpin structure", having a loop of 5 nt and a stem of 10 nt including five pairs of CG (Fig. 3). Like the typical E. coli terminators, this sequence structure is followed by a stretch rich in T residues (Rosenberg, M. and Court, D., Ann. Rev. Genet. 13, 314-353 (1979)).

Codon usage. Table 1 shows the codon usage of the H. influenzae iga gene. There is a striking tendency for the triplets to end preferentially with A or T. This may be a simple reflection of the high A+T content of the genome (mole percent A+T = 62). The codon frequencies in the iga gene are similar to those observed in four other H. influenzae genes for which the nucleotide sequence is known (Chandrasegaran, s. et al., Gene 70, 387-392 (1988)). A mole percent of 63% A+T was found for the 4626

nt H. influenzae iga gene sequenced, which is close to the published estimate of 62% A+T for the whole genome (Kilian, M., J. Gen. Microbiol. 93, 9-62 (1976)). According to the observations of Bibb et al., Gene 30, 157-166 (1984), genes of this genome should have an A+T content of 51%, 65%, and 69% for the first, second, and third position within codons. Values of 53%, 61%, and 75% A+T were found at these positions.

Based on the preference of T over C in the third base of duet codons and the relatively low quartet to duet frequency ratios among sextet codons encoding Arg, Leu, and Ser, the iga gene should be weakly expressed according to the rules of Grantham et al.,Nucl. Acids Res. 9, 43-74 (1981). This agrees well with the relatively low amount of IgA1 protease protein produced by H. influenzae.

This bias towards A and T residues of the third position of degenerate codons in the H. influenzae iga gene is in contrast to the markedly unbiased codon usage for the iga gene from N. gonorrhoeae which has a genomic A+T content of 51%.

These two genes most likely have a common ancestor indicating that apparently different constraints in codon usage have worked during the evolution of these two organisms.

Comparison of H. influenzae and N. gonorrhoeae protease sequences. Koomey and Falkow, Infect. Immun. 43, 101-1-7 (1984) have previously shown that the cloned type 2 N. gonorrhoeae iga gene hybridizes to the H. influenzae type 1 iga gene. The present comparison of the nucleotide sequences of these two iga genes revealed regions with a high degree of homology as well as areas with essentially no homology. The maximal homology between the

corresponding deduced amino acid sequences of the two IgA1 protease proteins was obtained by aligning them as shown in Fig. 4.

Polner et al., Nature 325, 458-462 (1987) found that the precursor of the N.gonorrhoeae IgA1 protease contains three functional domains; the amino-terminal signal peptide, the central IgA1 protease, and the carboxy-terminal "helper" domain. The leader peptide is released during translocation of the preprotein into the periplasmic space while the helper domain is assumed to create a pore in the outer membrane for extretion of the protease domain and remains associated with the membrane upon autoproteolytic cleavage.

The primary translation product, deduced from the H. influenzae iga sequence presented in Fig. 4, has a molecular mass of 169 kd. The mature type 1 IgA1 protease from H. influenzae has been estimated to be about 100 kd. Grundy et al. (J. Bacteriol. 169, 4442-4450 (1987)) found that a 2.2 to 3.1 kb region of the 3' end of a type 1 IgA1 protease gene from a H. influenzae serotype d strain is necessary for the secretion of the protease but not for its activity. They suggest that this region is cleaved off during maturation of the protease. Based on these observations and the amino acid sequence homologies shown in Fig. 4, it is suggested that the H. influenzae IgA1 protease is secreted by a mechanism similar to the one proposed by Polner et al., supra, for the N. gonorrhoeae IgA1 protease.

The aminoterminal part of the H. influenzae IgA1 protease contains positively charged lysines at amino acid (aa) positions 4, 5 and 7 followed by a hydrophobic stretch including the helix breaking proline at position 21 four residues before an alanine (Fig. 4). These features are

characteristic of signal sequences which are cleaved off and released with the alanine as the C-terminal amino acid, (Watson, M.E.E., Nucl. Acids Res. 12, 5145-5164 (1984)). The two protease sequences aligned as shown in Fig. 4 are identical around the position of the cleavage site of the N. gonorrhoeae protease leader peptide determined by Polner et al., supra. Therefore it is proposed that the 25 amino terminal amino acids of the H. influenzae IgA1 protease preprotein constitute the signal peptide.

Sequences rich in prolines, much like the type 1 IgA1 protease target site in the hinge region of the human IgA1 molecule (Fig. 1), are exclusively found at three positions in the H. influenzae protease sequence (A, B, and D in Fig. 4). A single sequence with homology to the type 2 IgA1 protease target site (Fig. 1) is found in the same region of the protease (C in Fig. 4) It is proposed that one or more of these four sequence elements are functionally equivalent to the autoproteolytic sites a, b, and c in the N. gonorrhoeae protease, as shown in Fig. 4.

When an N-terminal signal sequence of 25 amino acids is taken into account, autoproteolytic cleavage at positions A, B, C or D would result in excreted proteases of deduced molecular masses of 107.8, 108.1, 109.9, or 110.3 kd, respectively. Each of these values are in acceptable agreement with the estimated Mr of 100 kd.

Strikingly, a stretch of 32 amino acids (aa 16 to aa 47) is identical in the two proteases except for a single conservative substitution. This shows that the N-terminal part of the mature IgA1 protease has been evolutionary conserved suggesting that it is essential to the enzymatic function or specificity of the protease molecule.

Another notably well-conserved sequence is found at aa 785 to aa 797. It contains the two conserved cysteines which Polner et al.,supra, proposed to be part of the active site in the N.gonorrhoeae protease. A third cysteine is found in the "helper" domain of the H. influenzae protease preprotein at aa position 1250.

In striking contrast to the rest of the H. influenzae IgA1 protease, the region from aa 980 to aa 1240 has no significant sequence homology to the N. gonorrhoeae protease (see Fig. 4). This stretch is proposed to constitute the N-terminal part of the helper domain. It is very hydrophilic in both proteins suggesting that it has a common function in the secretion of the two proteases. No evolutionary deletions or insertions had to be introduced in this divergent area of the two protease sequences to obtain the flanking homologies as shown in Fig. 4. This observation is in contrast to the results of Grundy et al. who found a deletion-substitution loop in this region when analyzing DNA heteroduplexes formed between cloned H. influenzae type 1 and H. influenzae type 2 IgA1 protease genes.

The cloning and sequencing of the IgA1 protease gene from H. influenzae serotype b provides a tool to produce this protein in large quantities for further structural and functional analyses.

As mentioned, the IgA1 protease in question is primarily useful as an active component in vaccines for immunizing against bacterial meningitis. A vaccination against infections of the type concerned is in principle best performed by administering the vaccine via the oral cavity so that the vaccine gets into contact with the lymphoid tissues in the intestinal wall. Examples of administration of the vaccines are a) presentation on the surface of

transformed E.coli or other suitable host (vide infra), b) incorporated in micropheres alone or together with other suitable vaccine components or immunostimulant, and c) as part of a fusion protein with the subunit B of the cholera toxin. Administration of the vaccines may also be by the parenteral route. These examples are provided to describe and not to limit the possible applications.

The fact that the signals for expression of IgA1 gene and that the secretion mechanisms of the H. influenzae IgA1 proteases function in E. coli imply that the IgA1 protease gene has potential use as a vector for producing foreign proteins in cultures of E. coli or other suitable hosts.

The following examples are provided to describe this aspect in further detail. The examples are intended to illustrate and not to limit the invention:

Changes in the structural part of the iga gene do not affect secretion of the protein from the surface of the transformed E.coli clone. Insertion of prokaryotic or eukaryotic gene sequences encoding foreign polypeptides into IgA1 protease gene results in the transport to the E.coli cell surface of a hybrid protein. The hybrid protein may be exposed and retained on the cell surface or released by autoproteolysis into the growth medium depending on the location and size of the foreign gene insert. Such transformed E.coli clones or other suitable hosts exposing or releasing such fusion proteins on or from the cell surface may be used as an oral vaccine for presentation of one or more vaccine polypeptides to relevant lymphoid tissues in the gut of humans or animals. Alternatively, such clones may be employed for production of foreign polypeptides in E.coli or other hosts. Release into the medium may, when necessary, be accomplished by addition of active IgA1 protease.

Detailed Description of Aspect 2:

Bacterial strains

A collection of 133 isolates of Neisseria meningitidis recovered from 97 patients and 36 healthy carriers in 19 countries was examined. The 133 isolates represented 88 multilocus enzyme genotypes (ETs) defined on the basis of electrophoretically detectable allelic variations in 15 genes encoding enzymes (Caugant. d. et al., J. Bacteriol. 169, 2781-2792, 1987; Infect. Immun. 56, 2060-2068, 1988). In addition, 8 gonococcal strains, 4 each of the IgA1 protease types 1 and 2, were examined. IgA1 protease preparations produced by the method described by Higerd, T.B. et al. (J. Immunol. Methods 18, 245-249, 1977) from four meningococcal strains and one gonococcal strain were used to immunize rabbits by a method described previously (Kilian, M. et al. Molec. Immunol. 20, 1051-1058, 1983). The resulting antisera were examined for their ability to inhibit IgA1 proteases from all 133 meningococccal and 8 gonococcal strains using methods described (Kilian, M. et al., supra). While three of the four antisera against meningococcal proteases and the antiserum against gonococcal IgA1 protease inhibited IgA1 proteases from only some of the 141 strains, the remaining antiserum surprisingly caused inhibition of all IgA1 proteases whether of type 1 or 2 and whether of meningococcal or gonococcal origin. The inhibition titer determined against representative IgA1 protease preparations adjusted to standard activity as described (Kilian, M. et al. supra) ranged from 1024 to 4096. The meningococcal strain (HF13) used for production of this antiserum was of serogroup Y, subtype 2c, and produced an IgA1 protease of type 2. Identical IgA1 proteases were identified in other meningoccal strains of subtype 2c.

The IgA1 protease gene from Neisseria meningitidis strain HF13 was cloned in E.coli using methods described above for the cloning of the H.influenzae iga gene. A map of the gene and adjoining genome areas in phage lambda L47.1 is shown in fig. 5. The transformed E.coli clone secreted the meningococcal IgA1 protease into the growth medium and the resulting IgA1 protease isolated from the medium was capable of inducing neutralizing antibodies in rabbits with identical inhibition spectrum as antibodies raised against the IgA1 protease of the parental meningococcal strain HF13. These results show that the IgA1 protease isolated from meningococcal strain HF13, from a strain producing an identical or similar IgA1 protease, or from E.coli or other suitable host transformed with the iga gene, is a candidate for a vaccine against all types of meningococci and gonococci.

Detailed Description of Aspect 3:

The mentioned extensive IgA degradation in nasopharyngeal secretions of children with allergic diseases suggests that IgA1 protease-induced cleavage of IgA is a contributing factor to the development and perpetuation of allergic diseases. Two possible explanations might explain the more extensive IgA cleavage seen in allergic subjects: 1) an increased colonization by IgA1 protease-producing bacteria in the nasopharynx ad 2) an inability of allergy-prone subjects to react against bacterial IgA1 proteases with enzyme-neutralizing antibodies.

To study this, the nasopharyngeal flora of 24 children was examined at the age of 1 year and the age of approximately 30 months. Representative bacterial isolates were identified and examined for IgA1 protease activity using standard methods. All children were examined clinically

and immunologically to establish a possible diagnosis of allergic diseases.

Out of the 24 children, a total of 11 had developed allergic diseases at the age of 1 year. Table 1 correlates the clinically and immunologically established allergy diagnosis with the bacteriological findings. The figures demonstrate a significantly higher proportion of IgA1 protease-producing bacteria in nasopharyngeal samples from allergic children. Surprisingly, the numerically most significant IgA1 protease-producer was Streptococcus mitis biovar 1. This organism has previously been a member of "Streptococcus mitior" (Kilian, M., Mikkelsen, L., & Henrichsen, J. Int. J. Syst. Bacteriol. 39, 471-484, 1989). This organism occurs in an IgA1 protease-producing form and in a form not producing this enzyme. In accordance with the findings in the control group, previous studies have demonstrated that S.mitis biovar 1 normally occurs in the form lacking IgA1 protease activity in the nasopharynx (Kilian, M. & Holmgren, K. Infect. Immun. 31, 868-873, 1981). At the examination 18 months later, samples from allergic children contained Streptococcus pneumoniae and Streptococcus oralis in addition to bacteria listed in Table 1. These data demonstrate that children developing allergic diseases are colonized with IgA1 protease-producing bacteria in the nasopharynx to a higher extent than healthy children. A vaccine containing IgA1 proteases from the relevant bacteria mentioned is likely to secure that the immune barrier is left intact, thus preventing the development and perpetuation of allergic diseases.

TABLE 1

IgA1 protease producing bacteria as percentage of total bacterial flora in pharynx of 24 1 year old children with or without allergic diseases

		IgA1 protease-producing bacteria (% of total flora)				
	Subject	All species	H.influenzae	H.parahaemolyticus	S.mitis biovar 1	N.meningitidis
+Allergy	A	30%	0	0	30	0
	I	38%	2.4	0	33.4	2.2
	N	64%	1.0	0	63	0
	Å	36%	0	2	34	0
	X	52%	5.1	0	46.9	0
	a	58%	0.4	0	0	57.6
	D	14%	0	0	14	0
	O	38%	0	0	38	0
	R	18%	0	0	18	0
	Æ	31%	23	0	8	0
	Ø	22%	11.7	0	8.3	0
Median (range):		36% (14-64%)	0.4% (0-23%)	0 (0-2%)	30% (0-63%)	0 (0-57.6%)

TABLE 1 (continued)

IgA1 protease producing bacteria as percentage of total bacterial flora in pharynx of 24 1 year old children with or without allergic diseases

		IgA1 protease-producing bacteria (% of total flora)				
	Subject	All species	H.influenzae	H.parahaemolyticus	S.mitis biovar 1	N.meningitidis
-Allergy	K	8%	0.05	0	8	0
	M	6%	0	0	6.4	0
	P	4%	0	0	4	0
	T	2%	1.2	0	0.8	0
	Z	2%	0.1	0	1.9	0
	b	14%	0	3.6	10.4	0
	C	2%	0	0	0	2
	E	0.4%	0.4	0	0	0
	F	14%	2.1	0	11.9	0
	H	0.8%	0.8	0	0	0
	L	2%	0	0	2	0
	U	10%	0.4	0	9.6	0
	V	10%	1.1	2.2	6.7	0
Median (range):		4% (0.4-14%)	0.1% (0-2.1%)	0 (0-3.6%)	4% (0-10.4%)	0 (0-2%)

Explanation to the figures.

Fig. 1. Primary structure of the hinge region of human IgA1. The arrows indicate the peptide bonds cleaved by the individual IgA1 protease.

Fig. 2. Structure of the H. influenzae serotype b IgA1 protease gene. A Restriction maps of the analysed recombinant lambda phages containing sequences with homology to the H. influenzae serotype d iga gene probe. Symbols: (E) EcoRI; (H) HindIII; terminal bar indicates left arm of the individual phages; (+) or (-) indicate presence or absence of human IgA1 cleaving activity in culture supernatants of E. coli harbouring the individual clones. B Restriction fragments from EcoRI/HindIII double digests hybridizing to the two serotype d iga gene probes. The deduced orientation of the iga gene is indicated by 5' and 3'. C Combined restriction map of λ368iga-8 and λ369iga-16, an extension of A by including the restriction enzymes BamHI (B), ClaI (C), and PstI (P). D Restriction fragments of λ368iga-16 subcloned into M13mp19 for sequence analysis.

Fig. 3. Nucleotide sequence of the cloned iga gene from H. influenzae serotype b strain HK368. The sequence shown is derived from a part of the 7.5 kb EcoRI-HindIII fragment of the λ368iga-16 insert. The deduced amino acid sequence of the large ORF is shown above the nucleotide sequence. The two sequential stop codons are indicated by asterisks. Putative -35 and -10 promoter elements as well as a possible Shine-Dalgarno (SD) sequence are overlined. The possible Rho-independent dual terminator is indicated by divergent arrows.

Fig. 4. Amino acid homologies between the deduced H. influenzae iga gene product (H11GAP) and the N. gonorhoeae

iga gene product (NG1GAP). Sequence data of N. gonorrhoeae iga was derived from Pohlner et al., supra. Asterisks denote identical or functionally equivalent residues. Gaps indicated by dashes are introduced in the two sequences to obtain maximal homology. Cleavage site for the amino terminal signal peptide of the N. gonorrhoeae protease is indicated by s; S denotes the proposed equivalent cleavage site of the H. influenzae protease precursor. Positions a, b, and c represent autoproteolytic sites for the N. gonorrhoeae proteasse; positions A, B, C, and D indicate similar autoproteolytic sites suggested for the H. influenzae protease.

Fig. 5. Physical map of lambda HF13iga-1 expressing the meningococcal IgA1 protease gene from strain HF13. Symbols: E, Eco RI; H, Hind III; Xb, Xba I; Xh, Xho I. Hatched bar, fragment hydridizing to the 4.3 kb Hind III fragment of pVD 105 which contains the major part including the 3' end of the N.gonorrhoeae iga gene (Koomey, J.M., and Falkow, S., 1984 supra). The enzymes BamHi and SalI were found not to cleave the lambda HF13 iga-1 DNA.

C l a i m s:

1. Immunoglobulin A1 protease (IgA1 protease) and fragments thereof, c h a r a c t e r i z e d in that it is produced by cloning the gene for the IgA1 protease from a microorganism in a host organism, preferably selected among Escherichia coli, Salmonella and Saccharomyces, from which the IgA1 protease formed is secreted extracellularly.

2. IgA1 protease according to claim 1, c h a r a c t e r i z e d in that it is produced by cloning in an E. coli host organism.

3. IgA1 protease according to claim 1 or 2, c h a r a c t e r i z e d in that the cloned gene originates from Haemophilus influenzae serotype b.

4. Method of producing IgA1 protease according to claim 3, c h a r a c t e r i z e d in that an expression vector containing a gene coding for the IgA1 protease in question is introduced into an E. coli host organism, the gene originating from Haemophilus influenzae serotype b HK368, that the host organism is cultured to produce the IgA1 protease and that the protease is isolated from the culturing medium in a manner known per se.

5. Gene for use in an expression vector in the method according to claim 4, c h a r a c t e r i z e d in that it has the nucleotide sequence shown in Fig. 3, wherein SD represents a possible Shine-Dalgarno sequence.

6. Vaccine for immunizing against bacterial meningitis, c h a r a c t e r i z e d in that it contains IgA1 protease from Haemophilus influenzae serotype b or

fragments thereof according to claim 3, and optionally IgA1 protease from Neisseria meningitidis and/or Streptococcus pneumoniae.

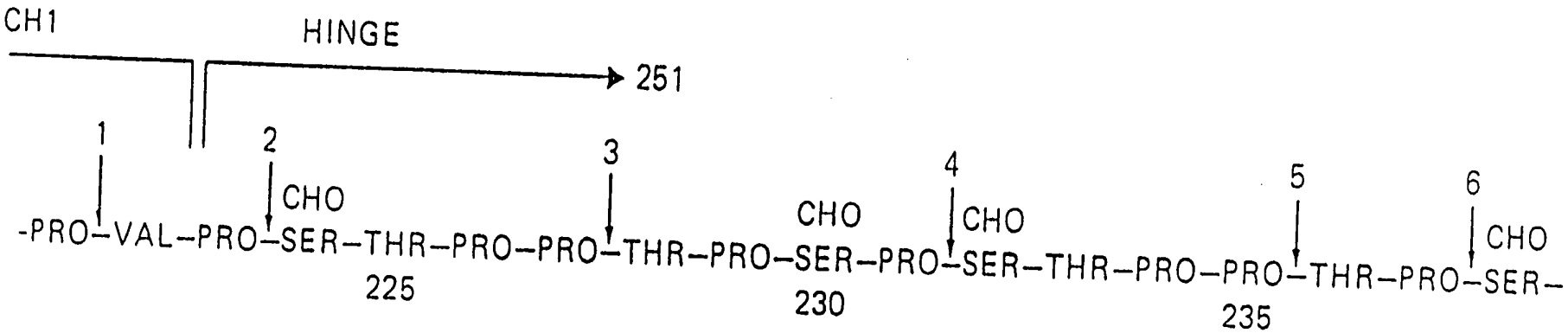
7. Vaccine according to claim 6, c h a r a c t e r - i z e d in that it is formulated for peroral use.

8. Vaccine for the prevention of gonorrhoea and meningococcal meningitis, c h a r a c t e r i z e d in that it contains IgA1 protease from Neisseria meningitidis.

9. Vaccine for the prevention or treatment of allergic diseases, c h a r a c t e r i z e d in that it contains IgA1 proteases from the bacteria which as a result of their cleavage of the mucosa antibodies are responsible for the allergic reaction.

10. Vaccine according to claim 9, c h a r a c t e r - i z e d in that the IgA1 proteases origine from bacteria selected among Haemophilus influenzae, H. parahaemolyticus, Streptococcus pneumoniae, Streptococcus sanguis, Streptococcus oralis, Streptococcus mitis biovar 1 and Neisseria meningitidis.

FIG. 1



1) *CLOSTRIDIUM RAMOSUM*

2) *BACTEROIDES MELANINOGENICUS*
CAPNOCYTOPHAGA OCHRACEA

3) *STREPTOCOCCUS PNEUMONIAE*
STREPTOCOCCUS SANGUIS
STREPTOCOCCUS ORALIS (»MITIOR»)
STREPTOCOCCUS MITIS

4) *HAEMOPHILUS INFLUENZAE*
HAEMOPHILUS AEGYPTIUS

5) *NEISSERIA GONORRHOEAE*
HAEMOPHILUS INFLUENZAE
NEISSERIA MENINGITIDIS

6) *NEISSERIA GONORRHOEAE*
NEISSERIA MENINGITIDIS

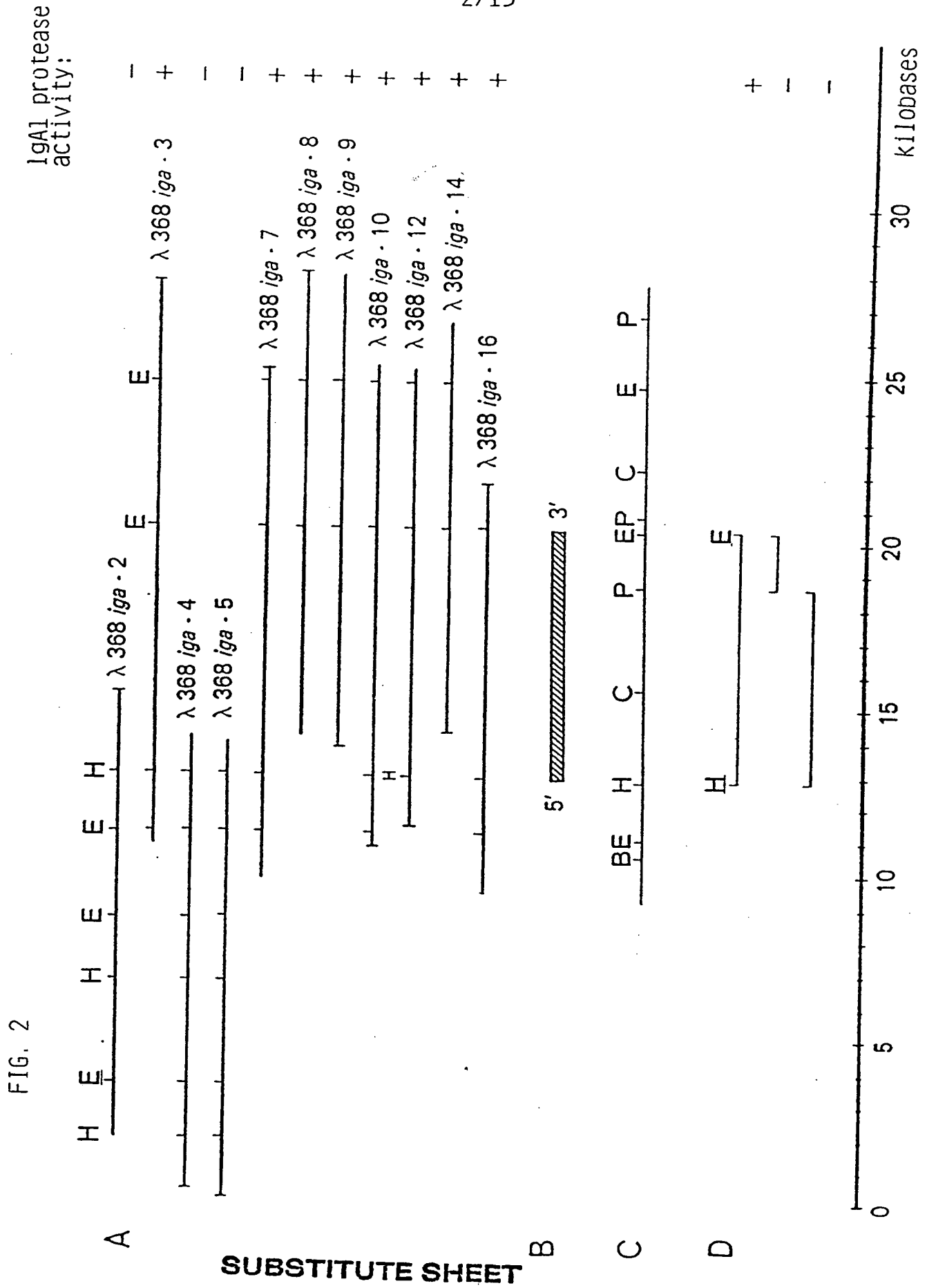

IgA1 protease activity:
λ 368 iga · 2
λ 368 iga · 3
λ 368 iga · 4
λ 368 iga · 5
λ 368 iga · 7
λ 368 iga · 8
λ 368 iga · 9
λ 368 iga · 10
λ 368 iga · 12
λ 368 iga · 14
λ 368 iga · 16
5'
3'
A
B
C
D
kilobases
0
5
10
15
20
25
30

FIG. 2

FIG. 3


```
CATCATTACGCCTTAAATTAACCAAAAGTGCGGTAAAAAATAGCCAATTTTTTAAAAAAT
      10        20        30        40        50        60

ATCAAAAATCATTATCATTACCTCGTAAATGTAATTCAAGTTTTGAGCGATTTATGCTAT
      70        80        90       100       110       120

AAAACCCACTCATTATAAAAATGAAGACAACTTGCAAACTATCAATGCAACACAGCCAAG
     130       140       150       160       170       180

                          -35                          -10
ATTTGAATCAACTTGTAGCCGTATATCAAATTGTGTCCTATCAAATCTACTTTTTAAACT
     190       200       210       220       230       240

SD
TAATTAATAAAGACAGCTTCTATGCTAAATAAAAAATTCAAACTCAATTTTATTGCACTT
     250       260       270       280       290       300
      M  L  N  K  K  F  K  L  N  F  I  A  L
T  U  A  Y  A  L  T  P  Y  T  E  A  A  L  U  A  D  D  U  D
ACTGTCGCCTACGCATTAACCCCTTATACAGAAGCCGCGTTAGTGAGAGACGATGTGGAT
     310       320       330       340       350       360
Y  Q  I  F  A  D  F  A  E  N  K  G  K  F  S  U  G  A  T  N
TATCAAATATTCCGTGATTTTGCAGAGAATAAAGGGAAGTTTTCTGTTGGTGCAACAAAT
     370       380       390       400       410       420
U  L  U  K  D  K  N  N  K  D  L  G  T  A  L  P  N  G  I  P
GTGCTGGTAAAGGATAAAAATAATAAAGATTTGGGCACTGCCTTACCTAACGGTATTCCG
     430       440       450       460       470       480
N  I  D  F  S  U  U  D  U  D  K  A  I  A  T  L  I  N  P  Q
ATGATTGATTTTAGCGTGGTAGATGTAGATAAACGCATTGCCACATTGATAAATCCACAA
     490       500       510       520       530       540
Y  U  U  G  U  K  N  U  S  N  G  U  S  E  L  N  F  G  N  L
TATGTAGTAGGTGTAAAACACGTTAGTAACGGCGTGAGTGAACTACATTTTGGTAACTTA
     550       560       570       580       590       600
N  G  N  N  N  N  G  N  A  K  A  N  R  D  U  S  S  E  E  N
AACGGCAATATGAATAATGGCAATGCCAAGGCACACCGAGATGTATCTTCAGAAGAAAAT
     610       620       630       640       650       660
```

FIG. 3 (continued)


```
 R  Y  F  S  U  E  K  N  E  Y  P  T  K  L  N  G  K  T  U  T
AGATATTTTTCCGTTGAGAAAAATGAGTATCCAACTAAATTGAATGGAAAAACAGTAACT
      670       680       690       700       710       720

 T  E  D  Q  T  Q  K  R  R  E  D  Y  Y  M  P  R  L  D  K  F
ACGGAAGATCAAACTCAAAAACGCCGTGAAGACTACTATATGCCACGTCTTGATAAATTT
      730       740       750       760       770       780

 U  T  E  U  A  P  I  E  A  S  T  A  S  S  D  A  G  T  Y  N
GTTACGGAAGTTGCACCAATAGAGGCTTCAACCGCAAGTAGTGATGCTGGCACATATAAT
      790       800       810       820       830       840

 D  Q  N  K  Y  P  A  F  U  A  L  G  S  G  S  Q  F  I  Y  K
GATCAGAATAAATATCCTGCTTTTGTAAGACTAGGAAGTGGTAGTCAATTTATTTATAAA
      850       860       870       880       890       900

 K  G  D  N  Y  S  L  I  L  N  N  H  E  U  G  G  N  N  L  K
AAAGGAGATAATTACAGCTTAATTTTAAATAATCATGAGGTTGGAGGCAATAATCTTAAA
      910       920       930       940       950       960

 L  U  G  D  A  Y  T  Y  G  I  A  G  T  P  Y  K  U  N  H  E
TTGGTGGGCGATGCCTATACCTATGGTATTGCAGGTACACCTTATAAAGTAAACCACGAG
      970       980       990      1000      1010      1020

 N  N  G  L  I  G  F  G  N  S  K  E  E  H  S  D  P  K  G  I
AATAATGGACTAATTGGTTTTGGCAATTCAAAAGAGGAACACAGCGATCCAAAAGGAATA
     1030      1040      1050      1060      1070      1080

 L  S  Q  D  P  L  T  N  Y  A  U  L  G  D  S  G  S  P  L  F
TTATCTCAAGATCCTCTTACCAATTATGCTGTTTTAGGCGACAGTGGCTCCCCATTATTT
     1090      1100      1110      1120      1130      1140

 U  Y  D  R  E  K  G  K  W  L  F  L  G  S  Y  D  F  W  R  G
GTATATGATAGAGAAAAAGGAAAATGGCTTTTTCTTGGGTCTTATGATTTTTGGGCGGGT
     1150      1160      1170      1180      1190      1200

 Y  N  K  K  S  W  Q  E  W  N  I  Y  K  S  Q  F  T  K  D  U
TATAACAAAAAATCTTGGCAAGAATGGAATATTTATAAATCTCAATTTACTAAAGATGTT
     1210      1220      1230      1240      1250      1260

 L  N  K  D  S  A  G  S  L  I  G  S  K  T  D  Y  S  W  S  S
CTCAATAAAGATAGTGCAGGTTCTTTAATTGGTTCCAAGACAGATTATAGTTGGTCTTCT
     1270      1280      1290      1300      1310      1320
```

FIG. 3 (continued)


```
 N  G  K  T  S  T  I  T  G  G  E  K  S  L  N  V  D  L  A  D
AATGGCAAGACAAGTACGATTACGGGAGGGGAGAAATCTTTAAATGTTGATTTAGCTGAC
      1330       1340       1350       1360       1370       1380

 G  K  D  K  P  N  H  G  K  S  V  T  F  E  G  S  G  T  L  T
GGAAAAGATAAACCTAATCACGGGAAAAGTGTTACATTTGAAGGGAGTGGAACGCTTACC
      1390       1400       1400       1420       1430       1440

 L  N  N  N  I  D  Q  G  A  G  G  L  F  F  E  G  D  Y  E  V
TTAAATAATAATATCGATCAAGGTGCAGGCGGATTATTCTTTGAAGGCGATTATGAAGTT
      1450       1460       1470       1480       1490       1500

 K  G  T  S  D  N  T  T  W  K  G  A  G  V  S  V  A  E  G  K
AAAGGTACTTCTGATAATACTACTTGGAAAGGAGCAGGTGTCTCTGTTGCCGAAGGAAAA
      1500       1520       1530       1540       1550       1560

 T  V  T  W  K  V  N  N  P  Q  Y  D  A  L  A  K  I  G  K  G
ACTGTAACGTGGAAAGTGCATAATCCTCAATATGATCGTTTAGCAAAAATTGGCAAAGGG
      1570       1580       1590       1600       1610       1620

 T  L  I  V  E  G  T  G  D  N  K  G  S  L  K  V  G  D  G  T
ACATTAATTGTTGAAGGAACAGGAGATAATAAAGGTTCGCTAAAAGTGGGCGATGGCACC
      1630       1640       1650       1660       1670       1680

 V  I  L  K  Q  Q  T  N  G  S  G  Q  N  A  F  A  S  V  G  I
GTTATTTTAAAACAACAAACAAATGGTTCGGGACAACACGCTTTTGCTTCTGTAGGGATT
      1690       1700       1710       1720       1730       1740

 V  S  G  A  S  T  L  V  L  N  D  D  K  Q  V  D  P  N  S  I
GTAAGTGGTCGCTCAACTCTTGTGCTTAATGATGATAAACAAGTAGATCCAAATTCAATT
      1750       1760       1770       1780       1790       1800

 Y  F  G  F  R  G  G  R  L  D  L  N  G  N  S  L  T  F  D  H
TACTTTGGCTTTAGAGGCGGTCGATTAGACTTAAACGGTAATTCACTAACCTTTGATCAC
      1810       1820       1830       1840       1850       1860

 I  R  N  I  D  D  G  A  R  L  V  N  N  N  M  T  N  A  S  N
ATCAGAAATATTGATGATGGTGCAAGACTAGTTAATCATAATATGACTAATGCCTCAAAT
      1870       1880       1890       1900       1910       1920

 I  T  I  T  G  E  S  L  I  T  D  P  N  T  I  T  P  Y  N  I
ATAACGATTACTGGGGAAAGTCTAATTACAGATCCAAATACAATTACTCCATATAATATA
      1930       1940       1950       1960       1970       1980

 D  A  P  D  E  D  N  P  Y  A  F  R  R  I  K  D  G  G  Q  L
GACGCACCAGATGAAGATAATCCTTATGCCTTTCGACGGATTAAAGATGGAGGACAGCTC
      1990       2000       2010       2020       2030       2040
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FIG. 3 (continued)


```
 Y  L  N  L  E  N  Y  T  Y  Y  A  L  R  K  G  A  S  T  R  S
TATTTAAATTTGGAAAATTACACTTATTATGCGTTAAGAAAAGGTGCGAGCACTCGTTCA
      2050       2060       2070       2080       2090       2100

 E  L  P  K  N  S  G  E  S  N  E  N  U  L  Y  M  G  K  T  S
GAATTACCTAAAAATAGTGGCGAAAGCAATGAAAATTGGCTATATATGGGTAAAACTTCC
      2110       2120       2130       2140       2150       2160

 D  E  A  K  R  N  U  M  N  H  I  N  N  E  R  M  N  G  F  N
GATGAAGCCAAAAGAAATGTAATGAACCATATCAACAACGAGCGTATGAATGGCTTTAAT
      2170       2180       2190       2200       2210       2220

 G  Y  F  G  E  E  E  G  K  N  N  G  N  L  N  U  T  F  K  G
GGTTATTTTGGCGAGGAAGAGGGTAAAAATAACGGTAATCTAAATGTGACTTTTAAAGGC
      2230       2240       2250       2260       2270       2280

 K  S  E  Q  N  R  F  L  L  T  G  G  T  N  L  N  G  D  L  T
AAAAGTGAGCAAAATCGCTTTTTATTAACAGGCGGTACAAACCTTAATGGCGATTTAACG
      2290       2300       2310       2320       2330       2340

 U  E  K  G  T  L  F  L  S  G  R  P  T  P  H  A  R  D  I  A
GTTGAAAAAGGCACCTTATTCCTTTCAGGCAGACCAACACCGCACGCAAGAGATATTGCA
      2350       2360       2370       2380       2390       2400

 G  I  S  S  T  K  K  D  P  N  F  A  E  N  N  E  U  U  U  E
GGTATTTCTTCGACAAAAAAAGATCCTCACTTTGCTGAAAATAATGAAGTGGTAGTAGAA
      2410       2420       2430       2440       2450       2460

 D  D  W  I  N  R  N  F  K  A  T  T  M  N  U  T  G  N  A  S
GATGACTGGATTAACCGCAATTTTAAAGCAACCACAATGAACGTGACTGGCAATGCCTCA
      2470       2480       2490       2500       2510       2520

 L  Y  S  G  R  N  U  A  N  I  T  S  N  I  T  A  S  N  K  A
CTTTATTCAGGTCGCAATGTTGCAAACATTACGTCAAATATCACAGCTTCTAATAAAGCA
      2530       2540       2550       2560       2570       2580

 Q  U  N  I  G  Y  K  T  G  D  T  U  C  U  R  S  D  Y  T  G
CAAGTTCATATCGGCTATAAAACAGGCGATACCGTTTGTGTACGTTCTGACTATACGGGC
      2590       2600       2610       2620       2630       2640

 Y  U  T  C  T  T  D  K  L  S  D  K  A  L  N  S  F  N  P  T
TATGTGACTTGTACTACTGACAAGTTATCCGATAAAGCCCTTAATAGCTTTAATCCAACC
      2650       2660       2670       2680       2690       2700

 N  L  R  G  N  U  N  L  T  E  S  A  N  F  U  L  G  K  A  N
AATCTACGCGGCAATGTAAATTTAACCGAAAGTGCAAACTTTGTCTTAGGCAAAGCCAAC
      2710       2720       2730       2740       2750       2760
```

```
 L  F  G  T  I  Q  S  R  G  N  S  Q  V  R  L  T  E  N  S  H
TTATTCGGCACAATTCAAAGCAGAGGAAATAGCCAAGTACGTTTAACCGAAAATAGCCAT
      2770       2780       2790       2800       2810       2820

 W  H  L  T  G  N  S  D  V  H  Q  L  D  L  A  N  G  H  I  H
TGGCATTTAACAGGAAATAGTGATGTTCATCAATTAGATCTAGCAAATGGGCATATTCAT
      2830       2840       2850       2860       2870       2880

 L  N  S  R  D  N  S  N  N  V  T  K  Y  N  T  L  T  V  N  S
TTAAATTCAGCAGACAATTCAAACAATGTGACAAAATATAACACGCTGACTGTGAATAGC
      2890       2900       2910       2920       2930       2940

 L  S  G  N  G  S  F  Y  Y  L  T  D  L  S  N  K  Q  G  D  K
TTATCAGGTAACGGTTCTTTCTATTATTTAACTGATCTTTCCAATAAACAAGGCGACAAA
      2950       2960       2970       2980       2990       3000

 V  V  V  T  K  S  R  T  G  N  F  T  L  Q  V  A  D  K  T  G
GTTGTTGTAACTAAATCCGCCACAGGTAACTTTACATTACAAGTGGCAGATAAAACAGGC
      3010       3020       3030       3040       3050       3060

 E  P  N  H  N  E  L  T  L  F  D  A  S  K  A  Q  A  D  N  L
GAGCCAAATCATAATGAACTCACACTTTTTGATGCTTCAAAAGCTCAAAGAGATCATTTG
      3070       3080       3090       3100       3110       3120

 N  V  S  L  V  G  N  T  V  D  L  G  A  W  K  Y  K  L  R  N
AATGTGTCATTAGTTGGGAATACCGTTGATTTAGGTGCTTGGAAATATAAATTACGTAAT
      3130       3140       3150       3160       3170       3180

 V  N  G  R  Y  D  L  Y  N  P  E  V  E  K  R  N  Q  T  V  D
GTTAATGGACGTTACGATTTGTATAACCCAGAGGTGGAAAAAGAAATCAAACTGTCGAT
      3190       3200       3210       3220       3230       3240

 T  T  N  I  T  T  P  N  N  I  Q  A  D  V  P  S  V  P  S  N
ACGACAAATATCACAACACCTAATAATATTCAAGCTGATGTGCCTAGCGTACCAAGTAAC
      3250       3260       3270       3280       3290       3300

 N  E  E  I  A  A  V  D  E  A  P  V  P  P  P  A  P  A  T  P
AATGAAGAAATAGCCCGTGTTGATGAAGCACCAGTTCCACCACCTGCGCCTGCTACACCA
      3310       3320       3330       3340       3350       3360

 S  E  T  T  E  T  V  A  E  N  S  K  Q  E  S  K  T  V  E  K
TCAGAGACAACTGAAACAGTGGCTGAAAATAGTAAGCAAGAAAGTAAAACAGTAGAGAAA
      3370       3380       3390       3400       3410       3420

 N  E  Q  D  A  T  E  T  T  A  Q  N  A  E  V  A  K  E  A  K
AACGAGCAAGACGCAACCGAGACAACAGCTCAAAATAGAGAAGTTGCAAAAGAAGCTAAA
      3430       3440       3450       3460       3470       3480
```

FIG. 3 (continued) 8/15

```
 S  N  U  K  A  N  T  Q  T  N  E  U  A  Q  S  G  S  E  T  K
TCAAATGTAAAAGCTAATACTCAAACAAATGAAGTAGCTCAAAGTGGAAGTGAAACCAAG
      3490       3500       3510       3520       3530       3540

 E  T  Q  T  T  E  T  K  E  T  A  T  U  E  K  E  E  K  A  K
GAAACTCAAACGACTGAAACAAAAGAAACAGCTACGGTAGAAAAAGAAGAAAAGGCTAAA
      3550       3560       3570       3580       3590       3600

 U  E  T  E  K  T  Q  E  U  P  K  U  T  S  Q  U  S  P  K  Q
GTAGAAACAGAGAAAACTCAAGAAGTCCCTAAAGTGACTTCTCAAGTGTCTCCGAAACAG
      3610       3620       3630       3640       3650       3660

 E  Q  S  E  T  U  Q  P  Q  A  E  P  A  A  E  N  D  P  T  U
GAACAGTCTGAAACTGTTCAACCGCAAGCAGAGCCTGCTCGTGAAAATGATCCGACTGTT
      3670       3680       3690       3700       3710       3720

 N  I  K  E  P  Q  S  Q  T  N  T  T  A  D  T  E  Q  P  A  K
AATATAAAAGAGCCTCAATCTCAAACAAATACAACAGCAGACACTGAACAACCTGCGAAA
      3730       3740       3750       3760       3770       3780

 E  T  S  S  N  U  E  Q  P  U  T  E  S  T  T  U  N  T  G  N
GAGACTAGCTCAAATGTTGAACAACCAGTGACAGAAAGCACAACAGTAAACACTGGAAAC
      3790       3800       3810       3820       3830       3840

 S  U  U  E  N  P  E  N  T  T  P  A  T  T  Q  P  T  U  N  S
TCTGTAGTGGAAAATCCAGAGAATACAACACCTGCTACAACTCAACCTACGGTTAATTCA
      3850       3860       3870       3880       3890       3900

 E  S  S  N  K  P  K  N  A  N  A  A  S  U  A  S  U  P  N  N
GAAAGCAGTAATAAGCCAAAGAATAGACATAGAAGAAGTGTTCGCTCAGTTCCGCATAAT
      3910       3920       3930       3940       3950       3960

 U  E  P  A  T  T  S  S  N  D  A  S  T  U  A  L  C  D  L  T
GTTGAACCAGCTACAACAAGTAGCAACGATCGTTCTACAGTAGCATTGTGCGATCTCACA
      3970       3980       3990       4000       4010       4020

 S  T  N  T  N  A  U  L  S  D  A  A  A  K  A  Q  F  U  A  L
AGTACAAACACAAATGCGGTACTTTCTGATGCAAGGGCAAAAGCACAATTTGTTGCATTA
      4030       4040       4050       4060       4070       4080

 N  U  G  K  A  U  S  Q  N  I  S  Q  L  E  M  N  N  E  G  Q
AATGTGGGGAAAGCAGTTTCTCAACATATTAGCCAGTTAGAAATGAATAACGAGGGGCAA
      4090       4100       4110       4120       4130       4140

 Y  N  U  W  U  S  N  T  S  M  N  K  N  Y  S  S  S  Q  Y  A
TATAACGTTTGGGTATCTAATACTTCAATGAACAAAAATTATTCCTCAAGTCAATATCGT
      4150       4160       4170       4180       4190       4200
```

```
 A  F  S  S  K  S  T  Q  T  Q  L  G  W  D  Q  T  I  S  N  N
CGTTTTAGTTCTAAAAGTACGCAAACTCAACTGGGTTGGGATCAAACAATCTCAAACAAT
      1210       1220       1230       1240       1250       1260

 V  Q  L  G  G  V  F  T  Y  V  R  N  S  N  N  F  D  K  A  T
GTTCAGTTAGGTGGCGTGTTTACTTATGTTCGCAATAGTAACAACTTTGATAAGGCAACA
      1270       1280       1290       1300       1310       1320

 S  K  N  T  L  A  Q  V  N  F  Y  S  K  Y  Y  A  D  N  N  W
AGTAAAAATACTCTAGCACAAGTTAATTTCTATTCTAAATATTATGCGGATAATCATTGG
      1330       1340       1350       1360       1370       1380

 Y  L  G  I  D  L  G  Y  G  K  F  Q  S  K  L  Q  T  N  N  N
TATTTGGGCATTGATTTAGGCTACGGCAAGTTCCAAAGCAAATTACAAACTAATCATAAT
      1390       1400       1410       1420       1430       1440

 A  K  F  A  A  N  T  A  Q  F  G  L  T  A  G  K  A  F  N  L
GCGAAATTTGCTCGCCATACTGCACAATTTGGTTTAACCGCAGGCAAAGCATTTAATCTT
      1450       1460       1470       1480       1490       1500

 G  N  F  G  I  T  P  I  V  G  V  R  Y  S  Y  L  S  N  A  D
GGCAATTTTGGTATTACGCCAATAGTAGGCGTGCGTTATAGCTATTTATCAAACGCTGAT
      1510       1520       1530       1540       1550       1560

 F  A  L  D  Q  A  A  I  K  V  N  P  I  S  V  K  T  A  F  A
TTTGCATTAGATCAAGCTCGCATTAAAGTAAATCCAATATCTGTCAAAACAGCCTTTGCT
      1570       1580       1590       1600       1610       1620

 Q  V  D  L  S  Y  T  Y  N  L  G  E  F  S  V  T  P  I  L  S
CAAGTTGATTTAAGTTATACTTATCACTTAGGCGAGTTTTCCGTTACGCCAATTTTGTCT
      1630       1640       1650       1660       1670       1680

 A  A  Y  D  A  N  Q  G  S  G  K  I  N  V  N  G  Y  D  F  A
GCTCGATATGATGCAAACCAAGGCAGCGGAAAAATTAATGTAAATGGATATGATTTTGCT
      1690       1700       1710       1720       1730       1740

 Y  N  V  E  N  Q  Q  Q  Y  N  A  G  L  K  L  K  Y  N  N  V
TACAACGTGGAAAACCAACAGCAATATAACGCAGGGCTTAAATTGAAATATCATAATGTG
      1750       1760       1770       1780       1790       1800

 K  L  S  L  I  G  G  L  T  K  A  K  Q  A  E  K  Q  K  T  A
AAATTAAGTCTAATAGGCGGATTAACAAAAGCGAAACAAGCGGAAAAACAAAAAACTGCA
      1810       1820       1830       1840       1850       1860
```

FIG. 3 (continued)


```
 E  L  K  L  S  F  S  F     *
GAATTAAAACTAAGTTTTAGTTTTTAATAAGCCTGTTTGAATTAACGTTATAAACAACAA
      1870       1880       1890       1900       1910       1920

AGCCCTGTGTATTACAGGGCTTTATTTTTGAATGAAATTCAGTGATTAAGTGCGGTGAAA
      1930       1940       1950       1960       1970       1980

AATCAGCGCATTTTTTATTTTTAACGTAAAAACGCTGGAATATTTTTCTCATATGCTGAG
      1990       5000       5010       5020       5030       5040

ATTTTGTCTTCGTGCTGAAGGGTTAAACCGATATTATCTAAACCGTTTAGC
      5050       5060       5070       5080       5090
```

FIG. 4

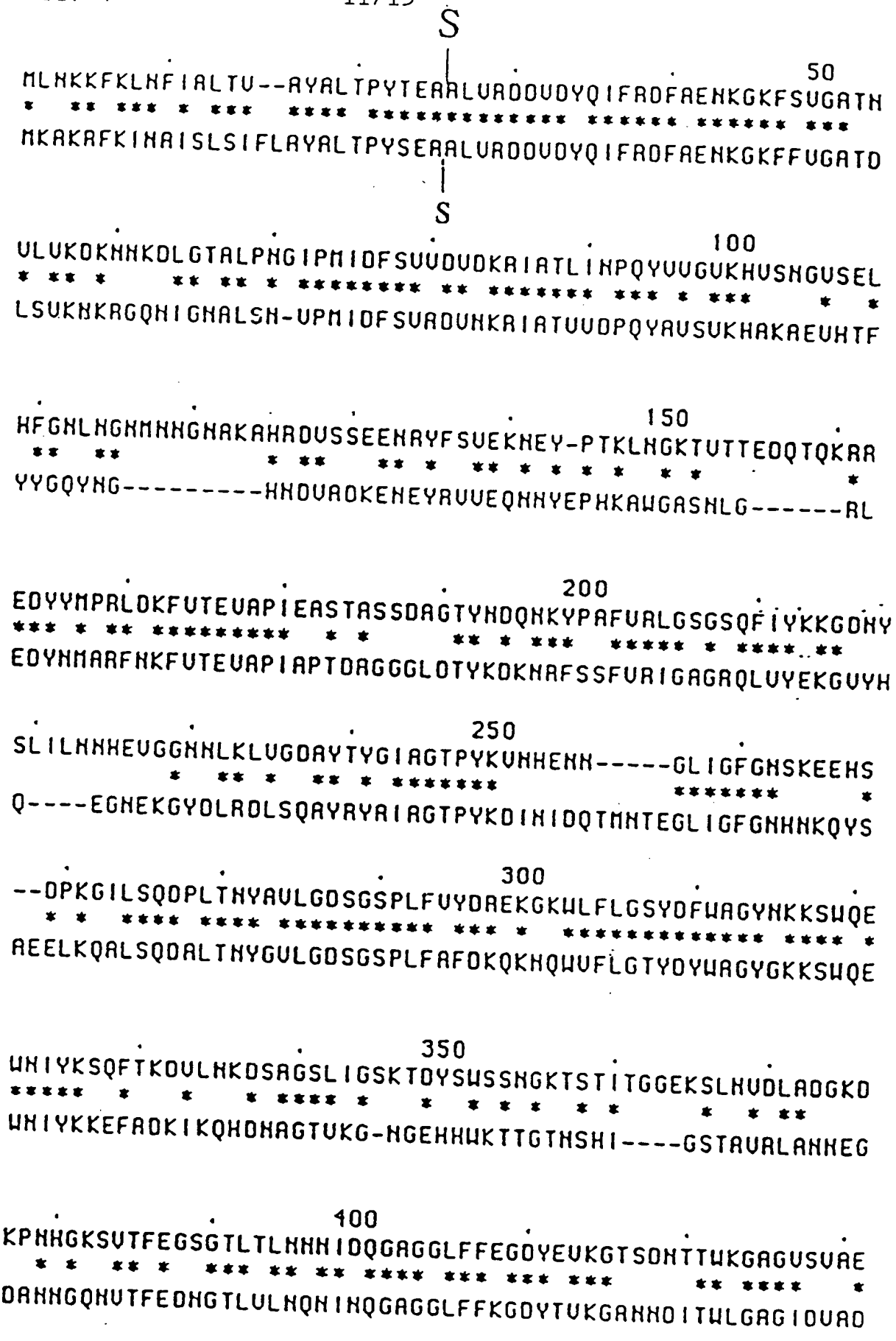

FIG. 4 (continued) 12/15

```
                  450
GKTVTWKVHNPQYDRLAKIGKGTLIVEGTGDNKGSLKVGDGTVILKQQTNGSGQH
** * * * * **    ** ****** * **  * * ******* ** *
GKKVVWQVKNPNGDRLAKIGKGTLEINGTGVNQGQLKVGDGTVILNQKRDRDKKV

          500
-AFASVGIVSGRSTLVLNDDKQVDPNSIYFGFRGGRLDLNGNSLTFDHIRNIDDG
 **  ******* *****   ** * ********** *** **********
QAFSQVGIVSGRGTLVLNSSNQINPDNLYFGFRGGRLDANGNDLTFEHIRNVDEG

       550
RRLVNNNMTNRSNITITGESLITDPNTITPYNI-DRPDEDNPYRF--RRIKDGGQ
*******   ** ***** **** *****   *    ***    *  *
RRIVNNNTDNRSTITLTGKSLITNPNSLSVNSIQNDYDEDDYSYYYRPRRPIPQG

     600
LYLNLENYTYYALRKGRSTRSELPKNSGESNENVLYMGKTSDEAKRNVNNNINNE
  *   ** ***** *       * *    * ***** * ****** *** **
KDLYYKNYRYYALKSGGRLNRPMPENGVAENNDWIFMGYTQEERRKNRMNNKKNNR

650                                                  700
RMNGFNGYFGEEEGK-NNGNLNVTFKGKSEQNRFLLTGGTNLNGDLTVEKGTLFL
*   * *** ** **  ** *** * *** ********* **** ***  * ***
RIGDFGGFFDEENGKGHNGALNLNFNGKSRQNRFLLTGGANLNGKISVTQGNVLL

                                                750
SGRPTPNRRDIRGISSTKKDPNFRENNEVVVEDDWINRNFKRTTMNVTGNRSLYS
**********    ** *** **  ***** ******* ***    *  **  *
SGRPTPNRRDFVNKSSRRKDRNFSKNNEVVFEDDWINRTFKRRETRVNQSRSFSS

                                                800
GRNVRNITSNITRSNKRQVNIGYKTGDTVCVRSDYTGYVTCTTDKLSDKRLNSFN
****  ** *****  * * **** ** ************ *  *********
GRNVSDITRNITRTDNRKVNLGYKNGDEVCVRSDYTGYVTCNTGNLSDKRLNSFD

                                          850
PTNLRGNVNLTESRNFVLGKRNLFGTIQSRGNSQVRLTENSNWNLTGNSDVNQLD
 * * ** *    * * ****  **     *** * *  *  ****  **
RTRINGNVNLNQNRRLVLGKRRLWGKIQGQGNSRVSLNQNSKWNLTGDSQVNNLS
```

FIG. 4 (continued) 13/15

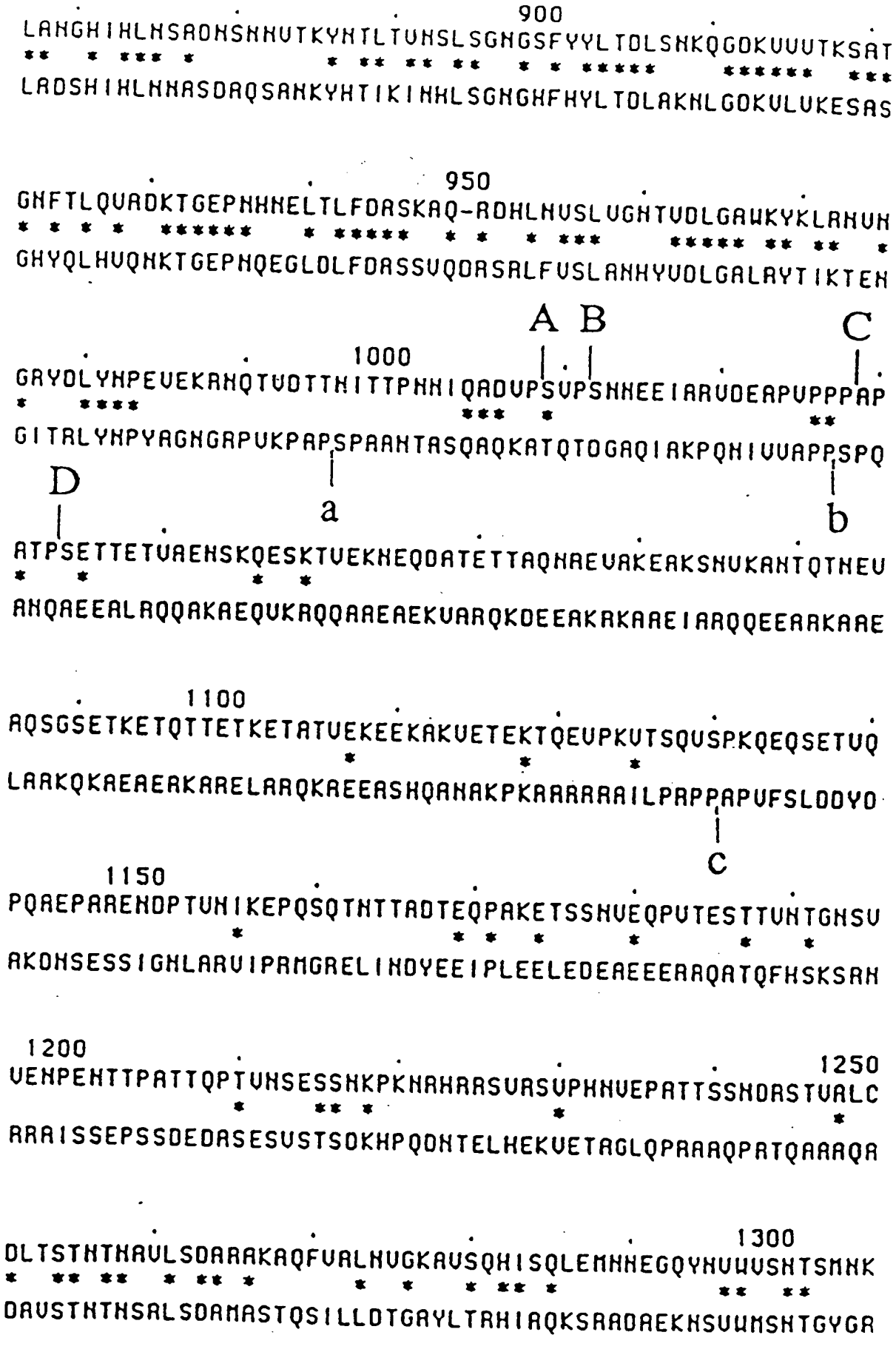

FIG. 4 (continued) 14/15

```
                                                    1350
HYSSSQYRRFSSKSTQTQLGWDQTISHNVQLGGVFTYVRHSHHFDKATSKHTLAQ
 * * ******** ****** * *** * ********      ** *  **** *
DYASAQYRRFSSKATQTQIGIDRSLSEHMQIGGVLTYSDSQHTFDQAGGKHTFVQ

                                          1400
VHFYSKYYADHHWYLGIDLGYGKFQSKLQTHHNAKFAAHTAQFGLTAGKAFHLGH
 *** ***    ***  *** * * *****  * * * * * *** * * *
AHLYGKYYLHDAWYVAGDIGAGSLASALQTQQKAHFHATSIQTGLTLGHTLKIHQ

                                    1450
FGITPIVGVRYSYLSHADFALDQAAIKVHPISVKTAFAQVDLSYTYHLGEFSVTP
* * *  ***** ** *** *    ***    ***  * *** * * ** *** *
FEIVPSAGIRYSRLSSADYKLGDDSVKVSSMAVKTLTAGLDFAYAFKVGHLTVKP

                                    1500
ILSAAYDANQGSGKIHVNGYDFAYHVENQQQYHAGLKLKYHNVKLSLIGGLTKAK
**** * ** * * *** *  ***  ****** *** * * ** * * * *** *
LLSAAYFAHYGKGGVHVGGKSFAYKADNQQQYSAGVALLYAHVTLHVNGSITKGK

          1540
QAEKQKTAELKLSFSF   HIIGAP
* ***** ***  *
QLEKQKSGQIKIQIAF   HGIGAP
```

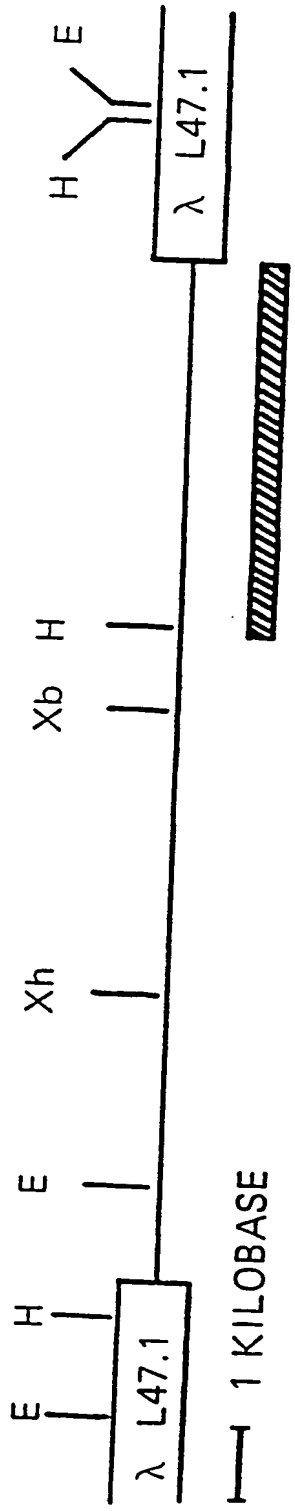

FIG.5

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 90/00073

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 P 21/02, C 12 N 9/50, C 12 N 15/57, A 61 K 39/02
39/095

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 N; C 12 P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Chemical Abstracts, volume 107, no. 21, 23 November 1987, (Columbus, Ohio, US), Grundy, Frank J. et al: "Haemophilus influenzae immunoglobulin A1 protease genes: cloning by plasmid integration-excision, comparative analyses, and localization of secretion determinants ", see page 207, abstract 192238f, & J.Bacteriol. 1987, 169(10), 4442-44509 --	1-5
X	Chemical Abstracts, volume 104, no. 15, 14 April 1986, (Columbus, Ohio, US), Fishman, Y et al: "Cloning of the type 1 immunoglobulin A1 protease from Neisseria gonorrhoeae and secretion of the enzyme from Escherichia coli ", see page 184, abstract 124204e, & Pathog.Neisseriae,Proc.Int.Symp.,4th 1984, 164(8), 9 --	1-6

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th June 1990	1990 -06- 26
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Anneli Jönsson

III. DOCUMENTS CONSIDERED T BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, volume 99, no. 5, 1 August 1983, (Columbus, Ohio, US), Bricker, J. et al: "IgA1 proteases of Haemophilus influenzae: cloning and characterization in Escherichia coli K-12 ", see page 132, abstract 33850z, & Proc.Natl.Acad.Sci.U.S.A. 1983, 80(9), 2681-26859 --	1-6
X	Chemical Abstracts, volume 98, no. 91, 28 February 1983, (Columbus, Ohio, US), Koomey, J. Michael et al: "Genetic and biochemical analysis of gonococcal IgA1 protease: cloning in Escherichia coli and construction of mutants of gonococci that fail to produce the activity ", see page 145, abstract 66414t, & Proc.Natl.Acad.Sci.U.S.A. 1982, 79(24), --	1-6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 90/00073

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 90-05-07
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date